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(71) Applicant *(for all designated States except US)*: BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SANICOLA-NADEL, Michele [US/US]; 4 Maple Road, Winchester, MA 01890 (US). HESSION, Catherine, A. [US/US]; 35 Otis Hill Road, Hingham, MA 02043 (US). WEI, Henry [CN/US]; 62 Farwell Street, Newton, MA 02160 (US). CATE, Richard, L. [US/US]; 64 Arrowhead Road, Weston, MA 02193 (US).

(74) Agent: FENTON, Gillian, M.; Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142 (US).

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(54) Title: MODULATORS OF TISSUE REGENERATION

(57) Abstract

Proteins which are upregulated in injured or regenerating tissues, as well as the DNA encoding these proteins, are disclosed, as well as therapeutic compositions and methods of treatment encompassing these compounds.

WAR cDNA
not mouse or human

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MODULATORS OF TISSUE REGENERATION

Related Applications

This is a continuation-in-part of prior U.S. Provisionals S.N. 60/047,490 and S.N. 60/047,491, both filed May 23, 1997. The teachings of both earlier-filed Provisional patent applications are incorporated herein by reference.

Field of the Invention

The invention relates generally to gene expression products, including nucleic acids (e.g., RNAs) and polypeptides that are upregulated in injured or regenerating tissues. Further, the invention relates generally to cDNAs and other nucleic acids encoding polypeptides that are upregulated in injured or regenerating tissues.

Background of the Invention

The processes of tissue development during embryogenesis, and of tissue repair and/or regeneration following injury or insult are presently the topic of intense investigation. Both processes involve a dynamic remodeling of tissue architecture, which is triggered and mediated by numerous biological interactions, including cell-cell contact, cell-matrix contact, release of soluble biological response modifiers, synthesis of structural components, and many other changes in cell phenotype, including changes in gene expression. Many of the factors involved in tissue genesis and in the response to tissue insult remain unknown or poorly understood.

Several systems have been developed for modeling the events triggered by insult to particular tissues, and/or by particular types of insult. For example, several investigators have described events occurring in mammalian kidney tissue exposed to an ischemia-reperfusion insult. The kidney is able to repair damage to the proximal tubule epithelium through a complex series of events involving cell death, proliferation of surviving proximal tubule epithelial cells, formation of poorly differentiated regenerative epithelium over the

denuded basement membrane, and differentiation of the regenerative epithelium to form fully functional proximal tubule epithelial cells (Wallin et al., Lab. Invest. 66:474-484, 1992; Witzgall et al., Mol. Cell. Biol. 13:1933-1942, 1994; Ichimura et al., Am. J. Physiol. 269:F653-662, 1995; Thadhani et al., N. Engl. J. Med. 334:1448-1460, 1996). Growth factors such as IGF, EGF, and HGF have been implicated in this process of repair, as has the endothelial cell adhesion molecule ICAM-1. However, the mechanisms by which the tubular epithelial cells are restored, either functionally and morphologically, remain poorly understood.

There accordingly is a need for an improved understanding of the biological processes of tissue repair or regeneration. That is, there is a need to identify factors that are indicative of the initiation, development and resolution of normal wound healing or normal biological response to tissue insult. Similarly, there is a need to identify factors that are indicative of pathologies of abnormal responses to tissue insult or other stimulus, including autoimmune or other dysregulation pathologies, as well as pathologies associated with abnormal tissue growth including neoplastic growth. Further, there is a need to identify factors that constitute appropriate targets for therapeutic intervention to stimulate, modulate, enhance, suppress or otherwise manipulate biological responses to tissue insult. Similarly, there is a need to identify factors that constitute appropriate targets for therapeutic intervention to manipulate abnormal or dysregulated responses to tissue insult or other stimulus, including abnormal tissue growth associated with cancer (neoplasia) and, conversely, abnormal tissue quiescence associated with degenerative diseases.

Summary of the Invention

It is an object of this invention to identify factors that are indicative of the initiation, development and resolution of normal wound healing or normal biological response to tissue insult. A specific object of this invention is to identify products (e.g., RNAs, cDNAs, polypeptides) of genes that are upregulated in the context of healing tissue, or in tissue exposed to insult or injury. A particular object is to identify products of genes that are upregulated in mammalian kidney tissue exposed to insult or injury, such as ischemia-

reperfusion injury. A second specific object of this invention is to identify products of genes that are upregulated in pathological conditions associated with abnormal responses to tissue insult or other stimulus, including autoimmune or other dysregulation pathologies, as well as pathologies associated with abnormal tissue growth including neoplastic growth. A third specific object is to identify gene products that constitute appropriate targets for therapeutic intervention to stimulate, modulate, enhance, suppress or otherwise manipulate biological responses to tissue insult. A particular object is to identify gene products for therapeutic intervention to manipulate responses of mammalian kidney tissue to tissue injury, such as ischemia-reperfusion injury. A fourth specific object is to identify gene products that constitute appropriate targets for therapeutic intervention to manipulate abnormal or dysregulated responses to tissue insult or other stimulus, including abnormal tissue growth associated with cancer (neoplasia) and, conversely, abnormal tissue quiescence associated with degenerative diseases. Thus, other particular objects of the invention include the identification of gene products as targets for therapeutic intervention in the clinical management (including prophylaxis, maintenance and treatment) of kidney diseases, including diseases involving renal failure, and of cancers derived from or affecting renal tissue.

The present invention rests on the discovery that the expression levels of numerous genes, including many genes heretofore unknown and/or uncharacterized, are upregulated in mammalian kidney tissue that has been exposed to tissue insult or injury. More specifically, the present invention rests on the discovery of a novel class of Kidney Injury-associated Molecules (each member of the class is henceforth called a "KIM"). KIMs are polypeptides encoded by genes whose expression levels are upregulated in the mammalian kidney upon exposure to tissue injury, particularly after exposure to ischemia-reperfusion injury. Any KIM is useful as an indicator of tissue status (preferably renal tissue status) or of a change therein (e.g, exposure to tissue injury, or the occurrence or stage of a tissue repair process). Any KIM further is useful as a target for therapeutic intervention to manipulate, whether by an agonist or an antagonist, a normal or abnormal tissue response to insult or other stimulus.

The invention accordingly provides, in a first aspect, purified and isolated nucleic acid molecules encoding all or a unique fragment of a KIM. In one embodiment, the present nucleic acids are RNAs. In another, they are DNA molecules, such as cDNAs. In another embodiment, the invention provides the complementary strands of nucleic acids encoding all or a unique fragment of a KIM. In another embodiment, the invention provides nucleic acids (preferably DNAs) that hybridize under low or, preferably, high stringency conditions to any of the foregoing nucleic acids. In still another embodiment, the invention provides nucleic acids (preferably DNAs) which, but for the degeneracy of the genetic code, would hybridize to any of the foregoing nucleic acids. In some embodiments, a nucleic acid encoding all or a unique fragment of a KIM is an engineered (recombinant) nucleic acid, optionally in operative association with an expression control element or other regulatory element. In other embodiments, a nucleic acid encoding all or a unique fragment of a KIM is an antisense nucleic acid sufficient, when internalized within a cell, to disrupt expression of a cellular KIM gene.

Specific novel KIMs (and unique fragments thereof) of the present invention are coded for by nucleic acids having the sequences disclosed herein in TABLE 1 and in the Sequence Listing. Other specific KIMs (and unique fragments thereof) are coded for by nucleic acids having sequences that are degenerate variants of any of the KIM sequences set forth in TABLE 1 and in the Sequence Listing. Still other specific KIMs (and fragments) are coded for by nucleic acids that are substantially similar to (homologous to) any of the KIM sequences in TABLE 1 and in the Sequence Listing. Such KIMs are defined herein as variants of the disclosed novel KIM sequences. In some embodiments, the present nucleic acid encodes a chimeric polypeptide comprising a novel KIM-encoding sequence (i.e., a sequence encoding part or all of a KIM) fused to a non-KIM sequence. Thus, the invention provides nucleic acids encoding novel KIM fusion proteins, non-limiting examples of which include KIM polypeptides fused to a secretable leader polypeptide, an immunoglobulin polypeptide, a binding pair partner (e.g., avidin, GST), a toxin or toxoid (e.g., ricin, tetanus), an enzyme (preferably one for which a detectable substrate is available, e.g., alkaline phosphatase, horseradish peroxidase, luciferase), or

other detectable polypeptide (e.g., green fluorescent protein). Additional specific KIMs (and unique fragments thereof) are coded for by nucleic acids referred to in TABLE 2. It is believed that, in most to all instances, these specific molecules have not heretofore been appreciated to be KIMs.

In a second aspect, the invention provides a vector having a KIM-encoding nucleic acid inserted therein. In some embodiments, the vector is a biologically functional plasmid or viral DNA vector. In other embodiments, the vector is a retroviral vector.

In a third aspect, the invention provides a prokaryotic or eukaryotic host cell comprising an internalized vector having a KIM-encoding nucleic acid insert. The present host cell provides intracellular means for producing (synthesizing, folding, processing, or secreting) a KIM polypeptide of the present invention.

In a fourth aspect, the invention provides a process for the production of a KIM polypeptide. The present production process includes growing a host cell of the invention under culture conditions sufficient for the production of polypeptides of vector origin, and recovering an expressed KIM polypeptide.

In a fifth aspect, the invention provides a purified and isolated novel KIM polypeptide, preferably substantially free of non-KIM polypeptides or proteins. In some embodiments, the present polypeptide is a full-length polypeptide, i.e., a polypeptide corresponding to the full-length open reading frame of a novel KIM-encoding cDNA. In other embodiments, the present polypeptide is a unique fragment of the full-length polypeptide. Thus, specific novel KIM polypeptides (and unique fragments thereof) of the present invention are coded for by nucleic acids having the sequences disclosed herein in TABLE 1 and in the Sequence Listing. Other specific novel KIM polypeptides of the present invention are variants of the disclosed novel KIM sequences, including without limitation splice variants, truncation variants, and substitution variants. In some embodiments, the invention provides a chimeric polypeptide comprising a novel KIM polypeptide fused (preferably via a peptide bond) to a non-KIM polypeptide. Thus, the invention provides novel KIM fusion proteins, exemplified by the above-mentioned fusion

constructs. Of course, the invention also provides conjugated or derivatized novel KIM polypeptides, including without limitation detectable conjugates, imageable conjugates, radiolabeled conjugates, and toxin conjugates. In still other embodiments, the invention provides chimeric polypeptides, fusion proteins and conjugates comprising a polypeptide appreciated herein as being a KIM (see TABLE 2).

In a sixth aspect, the invention provides an antibody that binds selectively to a KIM polypeptide. Preferably, the antibody is a monoclonal antibody (or an engineered derivative thereof) produced by conventional means from a hybridoma derived from splenocytes of an animal immunized with a KIM polypeptide of the present invention. In some embodiments, the present antibody is conjugated or derivatized with a detectable moiety, toxin, imageable compound or radionuclide.

In a seventh aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of a KIM nucleic acid (e.g., an antisense nucleic acid); a KIM vector; a KIM polypeptide; a KIM fusion protein; a KIM-binding antibody (also referred to as an anti-KIM antibody), dispersed, dissolved or otherwise suspended in a physiologically acceptable carrier, vehicle, solvent or excipient.

In an eighth aspect, the invention provides methods and kits for detecting, either qualitatively or quantitatively, KIM expression and/or KIM polypeptide. As disclosed herein, KIMs are upregulated by exposure of mammalian kidney (renal) tissue to insult or injury, particularly ischemia-reperfusion injury. Thus, any KIM (or combination or panel thereof) can be used as an indicator of tissue status (preferably renal tissue status), or of a change therein, including without limitation exposure to tissue injury, the occurrence or stage of an injury process, the occurrence or stage of a tissue repair or regeneration process, or the occurrence of an abnormal tissue response to injury or other stimulus, such as an autoimmune response or an abnormal proliferative response (e.g., a neoplastic response).

In one embodiment, the invention provides a detection method and kit for diagnosis, prognosis, staging, or monitoring of renal injury or of renal disease or of the effectiveness of therapy therefor. Some specific embodiments involve detecting and/or

measuring the concentration of one or more KIM polypeptides in serum, urine, or urine sediment of an individual (a mammal, preferably a human) afflicted with or at risk of developing renal injury or an impairment of renal function. Some such embodiments involve the use of at least one KIM polypeptide, anti-KIM antibody or conjugate thereof as elements of a reagent kit for immunoassay according to standard techniques. For present purposes, any KIM disclosed herein or identified as such herein can be used as a kit element, along with other elements for KIM detection. In other instances, such as where the detected KIM has biological or enzymatic activity, the kit can include reagents for detecting KIM activity, e.g., by enzyme assay. Other specific embodiments involve detecting and/or measuring the level of expression of one or more KIM-encoding nucleic acids in renal cells in a kidney biopsy, or in cells shed into urine or urine sediment of an individual afflicted with or at risk of developing renal injury or an impairment of renal function. Some such embodiments involve the use of a nucleic acid (e.g., RNA or DNA) encoding a KIM polypeptide, or a unique fragment thereof, or a probe nucleic acid capable of hybridizing to nucleic acid encoding a KIM, as elements of a reagent kit for hybridization assay according to standard techniques. As above, any KIM nucleic acid disclosed herein or appreciated herein as such can be used as a hybridization kit element, along with other reagents for detection of hybridized nucleic acids.

In another embodiment, the invention provides a method and kit for diagnosis, prognosis, staging or monitoring of an abnormal response of renal tissue to tissue injury or other stimulus, including an autoimmune response or an abnormal proliferative response, such as neoplasia arising from or affecting renal tissue. Some specific embodiments involve detecting and/or measuring the concentration of one or more KIM polypeptides in serum, urine, or urine sediment of an individual (a mammal, preferably a human) afflicted with or at risk of developing renal injury or an impairment of renal function. Some such embodiments involve the use of at least one KIM polypeptide, anti-KIM antibody or conjugate thereof as elements of a reagent kit for immunoassay according to standard techniques. For present purposes, any KIM disclosed herein or identified as such herein can be used as a kit element, along with other elements for KIM detection. In other instances,

such as where the detected KIM has biological or enzymatic activity, the kit can include reagents for detecting KIM activity, e.g., by enzyme assay. Other specific embodiments involve detecting and/or measuring the level of expression of one or more KIM-encoding nucleic acids in cells present in a kidney biopsy, or in cells shed into urine or urine sediment of an individual afflicted with or at risk of developing renal injury or an impairment of renal function. Some such embodiments involve the use of a nucleic acid (e.g., RNA or DNA) encoding a KIM polypeptide, or a unique fragment thereof, or a probe nucleic acid capable of hybridizing to nucleic acid encoding a KIM, as elements of a reagent kit for hybridization assay according to standard techniques. As above, any KIM nucleic acid disclosed herein or appreciated herein as such can be used as a hybridization kit element, along with other reagents for detection of hybridized nucleic acids.

In a ninth aspect, the invention provides a method and reagent for imaging tissues, either *in vitro* or *in vivo*. In particular, the invention provides a method and reagent for imaging the presence, extent or severity of kidney injury or of kidney tissue repair or regeneration. Similarly, the invention provides a method and reagent for imaging the presence, extent, severity or stage of an abnormal response to tissue injury or other stimulus, such as autoimmunity or neoplasia, particularly neoplasia arising from or affecting renal tissue. The present invention also provides a method for targetting an imageable compound to cells or tissue expressing or producing a KIM. The present method involves the step of contacting cells or tissue with a detectable (e.g., imageable) KIM-binding reagent, which in some embodiments is an anti-KIM antibody or conjugate thereof, or a KIM fusion protein. In other embodiments, the detectable KIM-binding reagent is a nucleic acid of the invention (e.g., a probe or antisense nucleic acid) labeled with a radionuclide or other imageable compound. According to the present method, cells expressing or producing a KIM are visualized (imaged) by detecting the presence and/or location of an accumulation of the KIM-binding reagent. For imaging *in vivo*, the KIM-binding reagent is administered, by any appropriate route, to an individual (a mammal, preferably a human) suspected of harboring an imageable locus of KIM expression and/or production. Without being limited hereby, it is believed that the present method can be

used to detect the presence and/or location of a tissue mass (e.g., a tumor) abnormally producing or expressing a KIM.

In a tenth aspect, the invention provides methods for treating an individual (a mammal, preferably a human) afflicted with or at risk of developing a disease or condition contributed to or associated with a dysfunction or dysregulation of a KIM gene or protein. Further, the invention provides methods for treating an individual afflicted with or at risk of developing a disease or condition beneficially affected by therapeutic administration of a KIM protein. The present methods involve the step of administering to the individual a therapeutically effective amount of a KIM polypeptide, variant or fusion protein thereof, or, conversely, of an anti-KIM antibody. It is expected that such compounds will be useful in therapeutic methods which manipulate, e.g., stimulate or inhibit, biological responses that are dependent on KIM function.

In an eleventh aspect, the invention provides a method for inhibiting the growth of KIM-expressing tumor cells, involving the step of contacting the cells with an anti-KIM antibody, conjugated to a toxin or radionuclide. In an alternative embodiment, the method involves the step of contacting the cells with an antisense KIM nucleic acid that is sufficient to suppress or disrupt expression of a KIM gene in the tumor cells.

In a twelfth aspect, the invention provides a method of gene therapy. The present method involves the administration of a vector capable of directing the production of a KIM, to an individual afflicted with or at risk of a renal disorder, disease or injury. As a result of the present method, growth of new tissue, preferably renal tissue, is stimulated, or survival of existing tissue, preferably renal tissue, is promoted.

The foregoing and other objects, features, aspects and advantages of the present invention, as well as the invention itself, will be more fully understood from the following description of preferred embodiments.

Detailed Description of the Invention

The discovery, reported herein, of a novel class of Kidney Injury-related Molecules (KIMs) was made by analyzing differences in mRNA expression between normal adult mammalian kidneys, and kidneys in the process of regenerating following exposure to tissue insult (specifically, to ischemia-reperfusion injury). Two established techniques were used for this purpose: representational difference analysis (RDA), and suppression subtractive hybridization (SSH). Both techniques were used to assess cDNAs isolated from various timepoints, e.g., 48 hours, after the onset of ischemia. In these studies, the normal adult kidney material was isolated from sham-operated subjects. Both techniques resulted in the depletion of cDNAs which are common to both postischemic and to normal kidney samples, leaving a pool of cDNAs which are significantly expressed only in injured or regenerating kidney tissue. This pool likely contains cDNAs corresponding to genes that encode proteins involved in the injury process, *and* proteins involved in tissue repair or regeneration processes. Therefore, cDNAs isolated from the pool likely encode parts or all of proteins likely to be therapeutically beneficial for treatment or prophylaxis of tissue injury, especially renal injury. Several cDNA clones have been obtained, sequenced and characterized.

Selected Definitions

A "KIM protein", herein used synonymously with "KIM", is any protein or polypeptide encoded by mRNA which is selectively upregulated following injury to a kidney. One group of KIM proteins of interest includes those coded for by mRNA which is selectively upregulated at any time within one week following any insult which results in injury to renal tissue. Examples of times at which such upregulation might be identified include 10 hours, 24 hours, 48 hours or 96 hours following an insult. Examples of tissue insults include toxin exposure, hypoxia, hyperoxia, hemodynamic disruption, ischemia, reperfusion, or mechanical compression. Many different types of proteins fall within the KIM class, including cell surface proteins (e.g., ligands or counter-receptors involved in cell-cell or cell-matrix interactions), secreted proteins (e.g., diffusable biological response modifiers, such as growth factors, differentiation factors, survival factors and the like),

intracellular proteins (e.g., elements of a signalling pathway), and nuclear proteins (e.g., transcription factors).

A "KIM ligand" is any molecule which noncovalently and specifically binds to a KIM protein. Such a ligand can be a protein, peptide, steroid, antibody, amino acid derivative, or other type molecule, in any form, including naturally-occurring, recombinantly produced, or otherwise synthetic. A KIM ligand can be in any form, including soluble, membrane-bound, or part of a fusion construct with immunoglobulin, fatty acid, or other moieties. The KIM ligand may be an integrin. A membrane-bound KIM ligand can act as a receptor which, when bound to or associated with KIM, triggers a cellular response. In some interactions, a KIM may associate with a plurality of KIM ligands, or may associate with a KIM ligand as part of a complex with one or more other molecules or cofactors. In a situation where both the KIM and the KIM ligand are bound to cell membranes, the KIM may associate and react with KIM ligand which is bound to the same cell as the KIM, or it may associate and react with KIM ligand be bound to a second cell. Where the KIM ligation occurs between molecules bound to different cells, the two cells may be the same or different with respect to cellular type or origin, phenotypic or metabolic condition, or type or degree of cellular response (e.g., growth, differentiation or apoptosis) to a given stimulus. "KIM ligation" refers to the contact and binding of KIM with a KIM ligand.

A "unique fragment" of a nucleic acid means any fragment of sufficient length to have a sequence likely to be substantially unique in a mammalian genome. Thus, a unique fragment generally means an oligonucleotide at least 16 nucleotide bases in length. Similarly, a "unique fragment" of a polypeptide means any fragment of sufficient length to have an amino acid sequence likely to be substantially unique to a given mammalian protein, such as a KIM. Thus, a unique peptide fragment generally means a peptide at least 7 amino acids in length.

A "KIM variant" means a KIM whose sequence differs from a sequence disclosed herein by the presence of one or more internal or terminal insertions, deletions or substitutions of a nucleotide (when referring to KIM nucleic acids) or of an amino acid

(when referring to KIM polypeptides). Preferably, the KIM variant is "substantially similar" to the corresponding disclosed KIM, or to a unique fragment thereof. That is, the KIM variant is "homologous" to the corresponding disclosed KIM. "Substantially similar" or "homologous" variants are structurally similar to the corresponding disclosed KIM. Further, "substantially similar" or "homologous" variants have sufficient functional similarity to the corresponding disclosed KIM that they share one or more of the KIM's biological properties or functions (e.g., binding to a receptor or ligand, triggering of biological responses, transport of a metabolite, catalysis of a substrate, or the like). A KIM variant can be naturally occurring or synthesized or produced by routine techniques, such as molecular engineering techniques.

By "alignment of sequences" is meant the positioning of one sequence, either nucleotide or amino acid, with that of another, to allow a comparison of the sequence of relevant portions of one with that of the other. Generally, sequences are aligned using the GAP and BESTFIT programs, which are based on the teachings of Needleman et al. (J. Mol. Biol. 48:443-453, 1970), Smith et al. (Adv. Appl. Math. 2:482-489, 1981), and Rechild et al. (CABIOS 5:107-113, 1989). Generally, when a homologous variant of a KIM is aligned with the corresponding KIM polypeptide, it will share amino acids that contribute to the KIM's three dimensional structure, such as cysteine residues. Although the relative positions of cysteine residues is generally conserved in the variant sequence, homologous or functionally equivalent sequences can include functionally equivalent arrangements of the cysteines, including arrangements comprising amino acid insertions or deletions which alter the linear arrangement of the cysteines, but do not materially impair their relationship or ability to form disulfide bonds in the folded structure of the KIM protein. Therefore, minor internal gaps and amino acid insertions, such as occur in splice variants, are ignored when aligning sequences herein.

"Sequence homology", "percent (%) homology", "sequence similarity" and "percent (%) similarity" are used interchangeably herein, and refer to the *sum* of the percentage of residues (whether nucleotides or amino acids) that are, when aligned with a reference sequence, the same as the corresponding reference residues, *and* those that are,

when aligned, conservative substitutions for the corresponding reference residues. "Sequence identity" and "percent (%) identity" also are used interchangeably herein, and refer to the percentage of residues (whether nucleotides or amino acids) that are, when aligned with a reference sequence, the same as the corresponding reference residues. Both sets of terms are used according to their definitions in Altschul et al. (1990), J. Mol. Biol. 215:403-410 and in the Basic Local Alignment Search Tool (BLAST) algorithm described therein. For present purposes, the algorithm gap weight is set at 3.0 and the length weight is set at 0.1.

"Hybridization" means the formation of a duplex nucleic acid molecule, in which nucleotide bases of a first polynucleotide strand bind noncovalently with cognate nucleotide bases of a second polynucleotide strand. Generally, such noncovalent binding occurrs in DNA only between adenosine (A) and thymidine (T) bases, and guanosine (G) and cytosine (C) bases. In RNA, binding occurs only between A and uracil (U), and G and C. The apposition of non-cognate bases in a duplex nucleic acid molecule, e.g., A with C, is termed a "mismatch" pairing. Under high stringency hybridization conditions, mismatches occur rarely in a given duplex nucleic acid molecule. Low stringency conditions permit the occurrence of some mismatches. Exemplary conditions which promote DNA hybridization, termed "hybridization conditions", include 6.0X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0X SSC at 50°C. The salt concentration in the wash step can be selected from a low stringency wash of about 2.0X SSC at 50°C, to a high stringency was of about 0.2X SSC at 50°C. In addition, the temperature in the wash step can be selected from a low stringency wash at room temperature, about 22°C, to a high stringency wash at about 65°C.

A "KIM agonist" is a molecule which can specifically trigger a cellular response normally triggered by the interaction of KIM with a KIM ligand. A KIM agonist can be a KIM variant, or a specific antibody to KIM, or a soluble form of the KIM ligand.

A "KIM antagonist" is a molecule which can specifically associate with a KIM ligand or a KIM, thereby blocking or otherwise inhibiting KIM binding to the KIM ligand. The antagonist binding blocks or inhibits cellular responses which would otherwise be

triggered by ligation of the KIM ligand with KIM or with a KIM agonist. Examples of KIM antagonists include certain KIM variants, KIM fusion proteins and specific antibodies to a KIM ligand or KIM.

"Isolated" refers to a condition in which a nucleic acid or polypeptide of the present invention is essentially free of other nucleic acids, polypeptides, or of other contaminants with which it might normally be found in nature, and as such exists in a form not found in nature.

"Substantially pure" refers to a condition in which a nucleic acid or polypeptide of the present invention is separated from other nucleic acids, polypeptides, or other contaminants, particularly naturally occurring contaminants, that interfere with the ability to detect, visualize or isolate the the nucleic acid or polypeptide of the invention, or that interfere with a biological function or property thereof. A substantially pure nucleic acid or polypeptide of the invention is not generally found in nature.

A "chemical derivative" of another molecule contains one or more additional chemical or biochemical moieties not found naturally in association with the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed, for example, in Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, Penn. (1980).

Other terms used herein, including "antisense DNA", "antisense probe", "cloning", "cDNA", "cDNA library", "DNA polymorphism", "expression", "gene", "hybridoma", "plasmid", "probe", "labeled", "recombinant", "host cells", "transformed", "transfected", "vector", and the like are used in the sense of their art-recognized meanings in the fields of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA or genetic engineering, and immunology. Such meanings are determined by consultation of one or more of the following widely available texts: Molecular Cloning, A Laboratory Manual, 2nd Ed. (Sambrook, Fritsch and Maniatis, eds.),

Cold Spring Harbor Laboratory Press, 1989; DNA Cloning, Volumes I and II (Glover, ed.), 1985; Oligonucleotide Synthesis (Gait, ed.), 1984; U.S. Patent 4,683,195, Mullis et al., invs.; Nucleic Acid Hybridization (Hames & Higgins, eds.), 1984; Transcription and Translation (Hames & Higgins, eds.), 1984; Culture of Animal Cells (Freshney), Alan R. Liss, publ., 1987; Immobilized Cells and Enzymes (IRL Press), 1986; A Practical Guide to Molecular Cloning (Perbal), 1984; Current Protocols in Molecular Biology, Wiley & Sons, publ., 1989; Methods in Enzymology, Academic Press, New York NY (especially Volumes 154 and 155); Gene Transfer Vectors for Mammalian Cells (Miller and Calos, eds.), Cold Spring Harbor Laboratory Press, 1987; Immunochemical Methods in Cell and Molecular Biology (Mayer and Walker, eds.), Academic Press, London, 1987; Handbook of Experimental Immunology, Volumes I-IV (Weir and Blackwell, eds.), 1986; and, Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, 1986.

Compounds of the Invention

Specific, novel KIMs discovered through RDA or SSH techniques are set forth below in TABLE 1 and in the Sequence Listing. TABLE 1 lists KIMs for which potential full length (PFL) cDNA clones were obtained. Also shown are the predicted polypeptide sequences encoded in the open reading frames (ORFs) of many of the newly discovered KIMs. In addition, TABLE 1 provides, where relevant, the subtracted amplified clone (SAC) designations and the PFL location(s) of partial sequences, many of which were reported in prior U.S. Provisional S.N. 60/047,490 and 60/047,491.

TABLE 1

SEQ. NO.	PFL	CLONE DESIGNATION	OPEN READING FRAME (OFR)	
			LOCATION	SEQ. ID #
# 3	HW011	HW011		
# 4	HW012	HW012	39. . 671	# 5
# 6	HW013	HW013		
# 7	HW014	HW014rev	175. . 804	# 8
# 9	HW015	HW15	11 . . 2176	# 10
# 12	HW017	SAC_24091		
# 13	HW018 HW101	SAC_23880	86 . . 1102	# 14
# 16	HW033	SAC_23901		
# 17	HW034	SAC_23897	91 . . 837 3101 . . 4162	# 18 # 19
# 20	HW035 HW112	SAC_24468		
# 21	HW036	SAC_24406		
# 22	HW037 HW102	SAC_24354	280 . . 1422	
# 26	HW040	SAC_24520	481 . . 2433	# 27
# 28	HW041	SAC_24317		
# 29	HW042 HW113	SAC_24017	8 . . 889	# 30
# 31	HW043	SAC_24533	8 . . 634	# 32
# 33	HW044	SAC_24216	24 . . 1100	# 34
# 35	HW045	SAC_24028	9 . . 1628	# 36
# 37	HW046	SAC_24036	9 . . 1160	# 38
# 39	HW047	SAC_23915	8 . . 511	# 40
# 42	HW050 HW109	SAC_24644	7 . . 1326	# 43
# 44	HW051	SAC_24170	114 . . 1505	# 45
# 46	HW052	SAC_24882	247 . . 765	# 47
# 49	HW055	SAC_24449	214 . . 1329	# 50
# 51	HW056	SAC_24326	8 . . 523	# 52
# 53	HW057	SAC_23926	7 . . 1023	# 54
# 55	HW059	SAC_24457	9 . . 1271	# 56
# 57	HW061	SAC_24029	7 . . 1422	# 58
# 61	HW069	SAC_24477	1360 . . 1893	# 62
# 64	HW073	SAC_24456	9 . . 1223	# 65
# 66	HW074	SAC_24464	7 . . 300	# 67
# 68	HW075 HW117	SAC_24466		
# 69	HW076	SAC_24409		
# 71	HW080	SAC_24033		
# 72	HW082	SAC_24469	150 . . 569	# 73
# 75	HW084	SAC_24854		
# 76	HW088	SAC_24336	9 . . 686	# 77
# 78	HW089	SAC_24461	8 . . 1006	# 79

TABLE 1, continued

SEQ. NO.	PFL	CLONE DESIGNATION	OPEN READING FRAME (OFR)	
			LOCATION	SEQ. ID #
# 80	HW090 HW118	SAC_24197	335 . 937	# 81
# 82	HW092	SAC_24320		
# 83	HW093	SAC_24538	8 . . 1357	# 84
# 85	HW094	SAC_23896	255 . . 1238	# 86
# 87	HW095	SAC_23802	7 . . 876	# 88
# 89	HW096	NONE	295 . . 1302	# 90

Additional KIMs, which are believed to be related to (e.g., substantially similar to) known molecules also were obtained through RDA or SSH techniques, and are set forth in TABLE 2 and in the Sequence Listing. It is believed that few to none of these molecules were previously appreciated to be KIMs, i.e., to have relevance to tissue injury or repair processes, particularly in the kidney. TABLE 2 lists these KIMs along with their clone designations. Partial sequences of some of the cDNA clones listed below were also set forth in the priority document. Also shown are the name(s) and GENBANK accession numbers of the known genes to which these KIMs may be related.

TABLE 2

SEQ 1D #	DESIGNATION	RELATED GENE	Accession #
92	23798	Rat alpha 1A (1D) adrenergic receptor	M60654
93	23800	Rat N27	V30789
94	23801	Rat alpha-2mu globulin-related protein	X13295
95	23833	Rat contrapsin-like protease inhibitor related protein	X16359
96	23836	Rat Fit-1	V04319
97	23853	Rat tumor-associated antigen (pE4)	L12025
98	23906	Rat sulfated glycoprotein 2	X13231
99	23907	Mouse secreted protein (p85)	L33416
100	23916	Human Differentiation-dependent A4 protein	L09604

TABLE 2, continued

SEQ 1D #	DESIGNATION	RELATED GENE	Accession #
101	23922	Rat manganese-containing superoxide dismutase	Y00497
102	24030	Rat heme oxygenase	J02722
103	24078	Rat tissue inhibitor of metalloprotein ase 1 (TIMP1)	U16022
104	24105	Rat alpha 2- macroglobulin	J02635
105	24140	Rat Cathepsin D	X54467
106	24142	Rat lysozyme	L12458
107	24192	Mouse MAMA mRNA	X67809
108	24296	Rat transketolase	U09256
109	24445	Rat heat stable antigen CD24/ Elongation factor 1	Z11531
110	24527	Cathepsin L	S85184
111	24540	Mouse endothelial monocyte-activating polypeptide 1	U41341
112	24623	Rat spleen thymosin beta4	K01334
1	HW010	Human semaphorin (CD100)	U60800
11	HW016	Mouse ribonucleotide reductase M2 subunit	M14223
15	HW032	Human S100C	D49355
24	HW038	Human splicing factor SF3a120	X85237
25	HW039	Annexin 11	M82802
41	HW049	Mouse u22 snoRNA host gene (UHG)	U40654
48	HW054	Rat myelin oligodendricyte protein (MOG)	M99485
59	HW062	Mouse proliferation-associated protein 1	U43918
60	HW066	Human cleavage stimulation factor 50kd subunit	L02547
63	HW070	Human BRCA2 region mRNA sequence CG037	U50523

TABLE 2, continued

SEQ ID #	DESIGNATION	RELATED GENE	Accession #
70	HW078	Human G protein gamma-10 subunit	P50151
74	HW083	Rat ins-1 winged helix	P97691
91	HW097	Human Na+ channel 2	U78181

Any of the foregoing KIM cDNAs can be inserted into a vector, liposome or other carrier vehicle for internalization and production in a host cell. Furthermore, the invention encompasses derivatives and variants of each of the foregoing KIMs as listed in TABLES 1 and 2.

One embodiment of the invention provides soluble variants of a KIM protein that is usually synthesized as a membrane associated protein. Soluble variants lack at least a portion of the transmembrane or intra-membrane section of a native KIM protein. In some examples, the soluble variant lacks the entire transmembrane or intra-membrane section of a native KIM protein. Soluble variants include fusion proteins which encompass derivatives of KIM proteins that lack at least a portion of the transmembrane or intra-membrane section of a native KIM protein. All types of KIM fusion proteins are included, particularly those which incorporate his-tag, Ig-tag, and myc-tag forms of the molecule. These KIM fusions may have characteristics which are therapeutically advantageous, such as the increased half-life conferred by the Ig-tag. Also included are fusion proteins which incorporate portions of selected domains of the KIM protein.

Variants can differ from a naturally occurring KIM protein in amino acid sequence or in ways that do not involve sequence, or both. Variants in amino acid sequence are produced when one or more amino acids in naturally occurring KIM protein is substituted with a different natural amino acid, an amino acid derivative or non-natural amino acid. Particularly preferred substitution variants include naturally occurring KIM proteins, or biologically active unique fragments thereof, whose sequences differ from the wild type sequence by one or more conservative amino acid substitutions, which typically have minimal influence on the secondary structure and hydrophobic nature of the protein or

peptide. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, such as substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Other conservative substitutions are those which meet the criteria for an "accepted point mutation" as defined in the Atlas of Protein Sequence and Structure (Dayhoff et al., eds.), 1978. See also PCT publication no. WO97/44460.

Substitution variants can also have sequences which differ by one or more non-conservative amino acid substitutions, provided however that the substitution does not abolish the native KIM protein's biological activity, ligand- or receptor-binding characteristics, or other functional property of interest. Exemplary non-conservative substitutions are those in which: (I) a hydrophilic residue, e.g., serine or threonine, is substituted for (or by) a hydrophobic residue, e.g., leucine, isoleucine, phenylalanine, or alanine; (ii) a cysteine residue is substituted for (or by) any other residue; (iii) a residue having an electropositive side chain, e.g., lysine, arginine or histidine, is substituted for (or by) a residue having an electronegative charge, e.g., glutamic acid or aspartic acid; or (iv) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

Other KIM variants of this invention include truncation variants (comprising at least a unique fragment of the corresponding KIM), insertion variants, and splice variants.

Other KIM variants within the invention are those with modifications which increase polypeptide stability. Such variants can contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of D- instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e.g., U.S. Patent 5,219,990.

KIM variants can be naturally-occurring, or produced through synthetic or molecular engineering techniques. Those of skill in the art will understand and appreciate that an engineered KIM variant can provide advantageous properties, e.g., in facilitating purification, improving stability, modulating a biological function, or the like. Thus, in some instances, a KIM variant will be desired that lacks a glycosylation site, or that has decreased aggregation potential due to elimination of a hydrophobic surface, or that has improved folding efficiency due to elimination of a cysteine residue, or the like.

In general, KIM variants have at least fifty (50) % amino acid sequence homology or similarity with the corresponding KIM protein. Preferably, the variants have at least sixty-five (65) % sequence similarity, more preferably at least eighty (80) % similarity with the corresponding KIM protein. Still more preferably, the sequence similarity is at least ninety (90) %, or most preferably, at least ninety-five (95) %. Other preferred KIM variants are those which 1) share at least forty (40) % similarity to the corresponding KIM protein, *and* 2) share at least eighty (80) % of aligned cysteine residues with the corresponding KIM protein.

Just as it is possible to replace substituents of the amino acid backbone or scaffold, it is also possible to replace, modify or add non-amino acid moieties to the scaffold. Such moieties can occur naturally in a given KIM as a result of post-translational processing, including acetylation, methylation, phosphorylation, carboxylation or glycosylation. As desired, such moieties can be removed or added by conventional synthetic or biochemical techniques. Further, non-natural moieties can be added to produce a derivative of a KIM or KIM variant of this invention. For example, polyethylene glycol (PEG) can be linked to a KIM to improve its stability or pharmacokinetic properties.

As will be readily appreciated, anti-KIM and anti-KIM variant antibodies can be produced by conventional techniques. Specifically contemplated are polyclonal and monoclonal antibodies, including antigen-binding fragments thereof and engineered derivatives thereof. Antigen-binding fragments of intact antibodies include complete Fab fragments, $F(ab')_2$ compounds, V_H regions, and F_V regions. Engineered derivatives of antibodies of the invention include single chain antibodies (see, e.g., WO 96/23071), as

well as human, humanized, primatized, or chimeric antibodies (see, e.g., PCT/US 95/16709). Engineered derivatives of the present antibodies are produced, generally, using standard recombinant DNA techniques (Winter and Milstein, Nature 349: 293-99, 1991). These include "chimeric" antibodies, in which the antigen binding domain from an animal antibody is linked to a human constant domain. In this instance, an antibody with the desired KIM-binding selectivity is derived initially from a nonhuman mammal (e.g., a mouse, rat or hamster), and subjected to recombinant DNA manipulation to replace all or part of the hinge and constant regions of the heavy chain and/or the constant region of the light chain, with corresponding regions from a human immunoglobin light chain or heavy chain. (See, e.g., Cabilly et al., United States Pat. No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. 81: 6851-55, 1984). Chimeric antibodies reduce the immunogenic responses elicited by animal antibodies when used in human clinical treatments.

In addition, recombinant "humanized" antibodies can be synthesized. Humanized antibodies are antibodies initially derived from a nonhuman mammal in which recombinant DNA technology has been used to substitute some or all of the amino acids not required for antigen binding with amino acids from corresponding regions of a human immunoglobin light or heavy chain. That is, they are chimeras comprising mostly human immunoglobulin sequences into which the regions responsible for specific antigen-binding have been inserted (see, e.g., PCT patent application WO 94/04679). Animals are immunized with the desired antigen, the corresponding antibodies are isolated and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of the human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (inter-species) sequences in antibodies for use in human therapies, and are less likely to elicit unwanted immune responses. Primatized antibodies can be produced similarly.

If desired, fully human antibodies with KIM-binding specificity which can be produced in nonhuman animals, such as transgenic animals harboring one or more human

immunoglobulin transgenes. Such animals may be used as a source for splenocytes for producing hybridomas, as is described in U.S. 5,569,825.

Identification of Specific KIMs of the Invention

To assess the dynamic biological processes of response to injury and tissue repair, a kidney ischemia-reperfusion model, which similates acute renal failure, was employed. The results of studies in this model system now have been published, and appear in Ichimura et al. (1998), J. Biol. Chem. 273:4135-4142, the teachings of which are incorporated by reference herein. In this model, the kidney has the capacity for cell renewal (both structurally and funcitonally) after injury to tubular epithelial cells. The nephron is damaged functionally by an ischemic reperfusion injury that results in regional areas of proximal tubule cell death. During the repair process, the kidney proximal tubule epithelium undergoes a complex series of events including (1) cell death and cast formation in the tubule lumen (casts are aggregates of dead, semiviable and viable cells, as well as of cell debris); (2) proliferation of surviving proximal tubule epithelial cells; (3) formation of a poorly differentiated regenerative epithelium over the denuded basement membrane (this simplified epithelium expresses vimentin, a mesenchymal marker); and (4) differentiation of the regenerative epithelium to form fully functional proximal tubule epithelial cells. Gene expression analysis of the kidney at various timepoints following the onset of ischemia revealed the upregulation of many KIMs anticipated to be involved in the injury and repair/regeneration processes.

1. Generation of ischemic and normal rat adult kidneys

Ischemic injured rat kidneys are generated as described by Witzgall et al. (J. Clin Invest. 93: 2175-2188, 1994). Briefly, the renal artery and vein from one kidney of an adult Sprague-Dawley rat are clamped for 40 minutes and then reperfused. Injured kidneys are harvested from the rats at 24 hours and at 48 hours after reperfusion. Kidneys from sham-operated, normal adult Sprague-Dawley rats are also harvested.

2. mRNA isolation

Post-ischemic rat kidneys are prepared as described by Witzgall et al. (J. Clin. Invest. 93: 2175-2188, 1994). Briefly, the renal artery and vein of the left kidney of an adult Sprague Dawley rat are clamped for 40 minutes and then reperfused. The ischemic kidneys are removed from rats either 24 hours or 48 hours after reperfusion. Normal rat kidneys are used as controls.

Total RNA is isolated with Trizol reagent (BRL catalog No. 1559-026). The kidney capsules are removed, then the kidneys are rinsed in PBS and immediately homogenized in Trizol solution. In some instances, the kidney is chopped into several pieces in PBS, frozen quickly in liquid nitrogen and kept at -70°C until it is processed for total RNA isolation. The mRNA is purified from total RNA according to the manufacturer's instructions, using an mRNA purification kit (Pharmacia, Catalog No. 27-9258-02).

3(i). Representational difference analysis (RDA) method to clone short-fragment cDNAs up-regulated by ischemia

The RDA method of Hubank and Schatz (Nucleic Acid Research 22:5640-48, 1994) is performed as described, with modifications as follow. Double-stranded cDNA is synthesized using the Superscript Choice® system (BRL Catalog No. 18090), from mRNA isolated from rat kidney either 24 hours or 48 hours post-ischemia as a tester and normal rat kidney as a driver. The tester and driver cDNA is digested with DpnII and ligated to R-Bgl-12/24 oligonucleotides. The adapter-ligated cDNA is amplified by PCR, and the PCR product is digested with DpnII to remove the oligonucleotides. The tester cDNA is ligated to J-Bgl-12-24 oligonucleotides.

Full length cDNA clones for Kim-1 and NMB contained in a plasmid vector are provided by T. Ichimura. They are digested with Sau3A1, purified and used as quenching reagents. cDNA fragments, 2-3 containing a fragment of annexin II, and 3-8 containing a fragment of alpha 2 macroglobulin (both provided by T. Ichimura) are amplified by PCR with R-Bgl-24 primer, digested with DpnII to remove the oligonucleotides and used as quenching reagents. Tester cDNA is hybridized to an excess driver cDNA including the four quenching reagents described above. After mung bean nuclease digestion.

hybridization mixture is amplified by PCR to enrich the cDNA fragments upregulated by ischemia. Each of the PCR steps is tested with a range of template DNA concentrations, and the concentration giving the best signal is chosen for the next steps. The DNA mixture containing tester and driver DNA for the subtractive hybridization step is washed twice with 70% EtOH by spinning for 3 minutes.

The second round cDNA subtraction is performed by hybridizing the first round cDNA subtraction product ligated to N-Bgl-12-24 oligonucleotides to an excess of the same driver cDNA used for the first round subtraction. The third round cDNA subtraction is performed by hybridizing the second round cDNA subtraction product ligated to J-Bgl-12/24 oligonucleotides to an excess of the same driver cDNA used for the first round of cDNA subtraction at a ratio of 1:14,000. The final PCR product after the third round of subtraction is digested with DpnII to remove the oligonucleotides and ligated to pCR-Script SK(+) vector. This represents a selected cDNA library by RDA.

3(ii). Suppression subtractive hybridization (SSH) method to clone short-fragment cDNAs up-regulated by ischemia

SSH is perfomed according to the manufacturer's instructions (Clontech, Catalog No. K1804-1) with certain modifications. Briefly, double-stranded cDNA are synthesized from mRNA isolated from rat kidney 24 hours or 48 hours post-ischemia as a tester, and from nomal rat kidney as a driver with the Superscript Choice™ system for cDNA synthesis (BRL Catalog No. 18090). The cDNA is synthesized according to the manufacturer's instruction except that the first strand cDNA is synthesized at 42°C. cDNA is digested with RSAI. Tester cDNA after RSAI digestion is ligated to adaptors. For the first hybridization, tester cDNA with adaptors is hybridized with excess driver cDNA at 68°C for 12 hours. For the second hybridization, the mixture is hybridized at 68°C for 22 hours. The cDNA mixture after the second hybridization is initially amplified by PCR for 27 cycles only. Then, a portion of the PCR product is re-amplified for 10 or 13 cycles. The final subtracted PCR product is digested by RSAI to remove the adaptor, and separated on a gel. The three bands and the remaining smear are cut out separately and cloned to pCR-

Script SK (+) vector (Stratagene, Catalog No. 211188). This represents a selected cDNA library by SSH.

4. Isolation and Characterization of Subtracted Amplified cDNA Clones

Colonies are randomly picked from the selected RDA and SSH cDNA libraries, and plasmid DNA is isolated with Qiagen plasmid kit. Each cDNA clone, referred to as a Subtracted Amplified Clone (SAC) is sequenced with the vector primers. DNA sequences are checked against GeneBank/EMBL databases for homology by the program BLAST™.

Southern blots are prepared with the initial PCR products generated from normal and injured rat kidneys. The blots are hybridized to the inserts isolated from the SACs to confirm which SACs are induced by ischemia.

4. Isolation and Characterization of potential full length cDNA clones

4.25 ug of polyA+ mRNA isolated from rat kidney 24 hours post-ischemia, which has been purified twice by oligo dT chromatography, is used to generate a cDNA library. In order to obtain double-stranded cDNA with EcoRI adaptors, a Superscript Choice® system for cDNA synthesis (BRL Catalog No. 18090) is used following the supplier's protocol, except that the cDNA synthesis reaction is carried out at 42°C. The DNA is ethanol precipitated, washed, resuspended in 5 ul H2O, and ligated to 2 ug EcoRI digested and CIAP-treated Lambda ZAPII (Strategene catalog No. 236612). The ligated DNA is packaged and used to infect E. coli XL-1 Blue MRF. The complexity of the library is $3.4x10^6$ independent recombinant plaques. Another cDNA library is generated from 4 ug of polyA+ RNA from rat kidney 48 hours post-ischemia as described above.

The two cDNA libraries described above are screened with inserts from the SAC clones. pBluescript plasmid vector containing cDNA inserts are excised from Lambda ZAPII vector by in vivo excision. Inserts from the longest cDNA clones, referred to as Potential Full Length (PFL) clones, are sequenced. DNA sequences are checked by the program BLAST™ against the GenBank/EMBL databases for DNA homology. Predicted protein coding regions (open reading frames, or ORFs) from some of the PFL clones also were used to search the GenBank/EMBL databases.

Diagnostic Uses of the Compounds of the Invention

Anti-KIM antibodies of the invention, which specifically bind to a protein of the invention or a unique fragment thereof, are useful in several diagnostic methods. These agents can be labeled with detectable markers, such as fluoroscopically or radiographically opaque substances, and administered to an individual to allow imaging of tissues which express KIM protein. The agents can also be bound to (conjugated to) substances, such as horseradish peroxidase, which can be used as immunocytochemical stains to allow visualization of areas of KIM protein-positive cells on histological sections. A specific antibody could be used alone in this manner, and sites where it is bound can be visualized in a sandwich assay using an anti-immunoglobulin antibody which is itself bound to a detectable marker.

Specific antibodies to KIM protein are also useful in immunoassays to measure KIM presence or concentration in samples of body tissues and fluids. Such concentrations may be correlated with different disease states. As an embodiment of particular interest, the invention includes a method of diagnosing renal injury, or of monitoring a process of renal repair, by measuring the concentration of KIM or of KIM fragments in the urine, plasma or serum of a patient. Similarly, KIM can be measured in urine sediment, in particular in cellular debris in the urine sediment. Casts of renal tubule cells, which may be present in urine sediment from patients with ongoing renal disease, may contain elevated levels of KIM protein and mRNA.

Specific antibodies to KIM protein may also be bound to solid supports, such as beads or dishes, and used to remove the ligand from a solution, either for measurement, or for purification and characterization of the protein or its attributes (such as post-translational modifications). Such characterization of an individual's KIM protein is expected to be useful in identifying deleterious mutants or processing defects which interfere with KIM function and are associated with abnormal phenotypes. Each of these techniques is routine to those of skill in the immunological arts.

Additional imaging methods utilize KIM or KIM fragments, fused to imageable moieties, for diagnostic imaging of tissues that express KIM ligands, particularly tumors.

Further diagnostic techniques are based on demonstration of upregulated KIM mRNA in tissues, as an indication of injury-related processes. (See, e.g., PCT publication no. WO 97/44460).

Therapeutic Uses of the Compounds of the Invention

The therapeutic methods of the invention involve selectively promoting or inhibiting cellular responses that are dependent on the presence or concentration of any KIM, including without limitation KIM ligation, KIM enzyme activity, KIM secretion, KIM signalling, and KIM modulation of gene expression. In instances where a KIM and a KIM ligand are both membrane bound, and expressed by different cells, the signal transduction may occur in the KIM-expressing cell, in the KIM ligand-expressing cell, or in both.

KIM ligation-triggered response in a KIM ligand-expressing cell may be generated by contacting the cell with exogenous KIM, KIM fusion proteins or activating antibodies against KIM ligand, either in vitro or in vivo. Further, responses of the KIM ligand-expressing cell that would otherwise be triggered by endogenous KIM could be blocked by contacting the KIM ligand-expressing cell with a KIM ligand antagonist (e.g., an antagonist antibody that binds to KIM ligand), or by contacting the endogenous KIM with an anti-KIM antibody or other KIM-binding molecule which prevents the effective ligation of KIM with a KIM ligand.

Similarly, the responses triggered by KIM ligation in the KIM-expressing cell may be promoted or inhibited with exogenous compounds. For example, KIM ligation-triggered response in a KIM-expressing cell may be generated by contacting the cell with a soluble KIM ligand, or certain anti-KIM activating antibodies. Further, responses of the KIM-expressing cell that would otherwise be triggered by interaction with endogenous KIM ligand could be blocked by contacting the KIM-expressing cell with an antagonist to KIM (e.g.., a blocking antibody that binds to KIM in a manner that prevents effective, signal-generating KIM ligation), or by contacting the endogenous KIM ligand with an anti-

KIM ligand antibody or other KIM ligand-binding molecule which prevents the effective ligation of KIM with the KIM ligand.

Which of the interventions described above are useful for particular therapeutic uses depend on the relevant etiologic mechanism of either the pathologic process to be inhibited, or of the medically desirable process to be promoted, as is apparent to those of skill in the medical arts. For example, where KIM ligation results in desirable cellular growth, maintenance of differentiated phenotype, resistance to apoptosis induced by various insults, or other medically advantageous responses, one of the above-described interventions that promote ligation-triggered response may be employed. In the alternative, one of the inhibitory interventions may be useful where KIM ligation invokes undesirable consequences, such as neoplastic growth, deleterious loss of cellular function, susceptibility to apoptosis, or promotion of inflammation events.

Following are examples of the previously described therapeutic methods of the invention. One therapeutic use of the KIM-related compounds of the invention is for treating a subject with renal disease, promoting growth of new tissue in a subject, or promoting survival of damaged tissue in a subject, and includes the step of administering to the subject a therapeutically effective amount of a KIM protein of the invention, or of a pharmaceutical composition which includes a protein of the invention. The protein used in these methods can be a unique fragment of a full-length KIM protein, a soluble KIM variant or a soluble ligand thereof, a KIM fusion protein, or a KIM agonist. These methods can also be practiced by administering to the subject a therapeutically effective amount of an agonist antibody of the invention, or a pharmaceutical composition which includes an agonist antibody of the invention. A KIM protein can be administered concurrently with a therapeutically effective amount of a second compound which exerts a medically desirable adjunct effect. While tissues of interest for these methods include any tissue, preferred tissues include renal tissue, liver, neural tissue, heart, stomach, small intestine, spinal cord, or lung. Particular renal conditions which are expected to be beneficially treated with the compounds of the invention include acute renal failure, acute nephritis, chronic renal failure, nephrotic syndrome, renal tubule defects, kidney

transplants, toxic injury, hypoxic injury, and trauma. Renal tubule defects include those of either hereditary or acquired nature, such as polycystic renal disease, medullary cystic disease, and medullary sponge kidney. This list is not limited, and may include many other renal disorders (see, e.g., Harrison's Principles of Internal Medicine, 13th ed., 1994, which is herein incorporated by reference.) The subject of the methods may be human.

A therapeutic intervention for inhibiting growth of undesirable, KIM- or KIM-ligand-expressing tissue in a subject includes the step of administering to the subject a therapeutically effective amount of a KIM antagonist (e.g.., an antibody blocks KIM ligation). In an embodiment of interest, the KIM antagonist or anti-KIM antibody can be used therapeutically to inhibit or block growth of tumors which depend on KIM protein for growth.

Other methods of the invention include killing KIM ligand-expressing tumor cells, or inhibiting their growth, by contacting the cells with a fusion protein of a KIM and a toxin or radionuclide, or an anti-KIM ligand antibody conjugated to a toxin or radionuclide. The cell can be within a subject, and the protein or the conjugated antibody is administered to the subject.

Also encompassed within the invention is a method for targeting a toxin or radionuclide to a cell expressing a KIM, comprising contacting the cell with a fusion protein comprising a KIM ligand and a toxin or radionuclide, or an anti-KIM antibody conjugated to a toxin or radionuclide. Another embodiment includes the method of suppressing growth of a tumor cell which expresses KIM, comprising contacting the cell with a fusion protein of KIM ligand and a toxin or radionuclide or with an anti-KIM antibody conjugated to a toxin or radionuclide; the cell may be within a subject, and the protein administered to the subject.

The term "subject" as used herein is taken to mean any mammal to which KIM can be administered. Subjects specifically intended for treatment with the method of the invention include humans, as well as nonhuman primates, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice.

Use of Compounds of the Invention in Gene Therapy

The KIM genes of the invention are introduced into damaged tissue, or into tissue where stimulated growth is desirable. Such gene therapy stimulates production of KIM protein by the transfected or transformed cells, promoting cell growth and/or survival of cells that express the KIM protein.

In a specific embodiment of a gene therapy method, a gene coding for a KIM protein is be introduced into a renal target tissue. The KIM protein is expected to be stably expressed and stimulate tissue growth, division, or differentiation, or to potentiate cell survival. Furthermore, a KIM gene can be introduced into a target cell using a variety of well-known methods that use either viral or non-viral based strategies.

Once introduced into a target cell, sequences of interest can be identified by conventional methods such as nucleic acid hybridization using probes comprising sequences that are homologous/complementary to the inserted gene sequences of the vector. In another approach, the sequence(s) may be identified by the presence or absence of a "marker" gene function (e.g, thymidine kinase activity, antibiotic resistance, and the like) caused by introduction of the expression vector into the target cell.

Formulation

In general, compounds of the invention are suspended, dissolved or dispersed in a pharmaceutically acceptable carrier or excipient. The resulting therapeutic composition does not adversely affect the subject's homeostasis, particularly electrolyte balance. Thus, an exemplary carrier comprises normal physiologic saline (0.15M NaCl, pH 7.0 to 7.4). Other acceptable carriers are well known in the art and are described, for example, in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., 1990. Acceptable carriers can include biocompatible, inert or bioabsorbable salts, buffering agents, oligo- or polysaccharides, polymers, viscosity-improving agents, preservatives, and the like. In some embodiments, the term "carrier" encompasses liposomes and the HIV-1 tat protein (See Chen et al., Anal. Biochem. 227: 168-175, 1995) as well as any plasmid and viral expression vectors.

Any KIM compound of this invention can be used in the form of a pharmaceutically acceptable salt. Suitable acids and bases which are capable of forming salts with the polypeptides, nucleic acids and vectors of the present invention are well known to those of skill in the art, and include inorganic and organic acids and bases.

KIM compounds of the invention are dispersed in the carrier to concentrations sufficient to deliver to the subject a therapeutically effective amount of the compound, which is an amount sufficient to produce a detectable, preferably medically beneficial effect in the subject. Medically beneficial effects would include preventing, delaying or attenuating deterioration of, or detectably improving, the subject's medical condition. It is expected that the concentration or amount of a KIM compound that will produce a medically beneficial effect will vary considerably with the circumstances in which the invention is practiced. An effective amount can be determined by an ordinarily skilled physician or other practitioner through no more than routine experimentation. As an example, an indication of the status of renal injury or renal function can be monitored with one or more routine laboratory tests which measure the concentrations of relevant substances in blood or urine, other urine characteristics, or the rate of clearance of various substances from the blood into the urine. The parameters measured by these tests, either individually or in combination, can be used by a physician to assess renal function or damage. Examples of such parameters include the blood concentration of urea, creatinine or protein; the urine concentration of protein or of various blood cells such as erythrocytes or leucocytes; urine specific gravity; amount of urine; the clearance rates of inulin, creatinine, urea or ρ-aminohippuric acid; and the presence of hypertension or edema.

In some embodiments, a KIM compound is formulated in a liposome delivery system, including without limitation any of a variety of unilamellar vesicles, multilamellar vesicles, or stable plurilamellar vesicles, all of which can prepared and administered according to methods well known to those of skill in the art, for example in accordance with the teachings of United States Patent 5,169,637, 4,762,915, 5,000,958 or 5,185,154. In addition, it may be desirable to express the novel polypeptides of this invention, as well as other selected polypeptides, as lipoproteins, in order to enhance their binding to

liposomes. As an example, treatment of human acute renal failure with liposome-encapsulated KIM protein may be performed in vivo by introducing a KIM protein into cells in need of such treatment using liposomes. The liposomes can be delivered via catheter to the renal artery. The recombinant KIM protein is purified, for example, from CHO cells by immunoaffinity chromatography or any other convenient method, then mixed with liposomes and incorporated into them at high efficiency. The encapsulated protein may be tested in vitro for any effect on stimulating cell growth.

Routes of Administration

The compounds of the invention may be administered in any manner which is medically acceptable. Depending on the specific circumstances, local or systemic administration may be desirable. Preferably, the compound is administered via a parenteral route such as by an intravascular, intravenous, intraarterial, subcutaneous, intramuscular, intratumor, intraorbital, intraventricular, intraperitoneal, subcapsular, intracranial, intraspinal, or intranasal injection, infusion or inhalation. The compound also may be administered by implantation of an infusion pump, or a biocompatible or bioerodable sustained release implant, or by installation of a catheter (e.g., in a renal artery), into the subject. Alternatively, certain compounds of the invention, or formulations thereof, may be appropriate for oral or enteral administration. Still other compounds of the invention will be suitable for topical administration.

Treatment Regimes

Determining appropriate dosage and frequency of treatment with any particular KIM compound to be administered to an individual is within the skills and clinical judgement of ordinary practitioners. The general dosage and treatment schedule is established by preclinical and clinical trials, which involve extensive but routine studies to determine the optimal administration parameters of the compound. Even after such recommendations are made, the practitioner will often vary these dosages for different individuals based on a variety of considerations, such as the individual's age, medical status, weight, sex, and concurrent treatment with other pharmaceuticals. Determining the

optimal dosage and administration regime for each KIM compound is a routine matter for those of skill in the pharmaceutical and medical arts.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative of, rather than limiting on, the invention disclosed herein. Scope of the invention thus is indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: HW059

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 9..1271

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1991..2162
(D) OTHER INFORMATION: /label= SAC_24457

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

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GTCGACAG CCC GCC ACT CCA GGC GCG ATG CTG TTC TGG ACT GTG CTC AGC          50
         Pro Ala Thr Pro Gly Ala Met Leu Phe Trp Thr Val Leu Ser
           1               5                  10

ATG GCT TTG AGT CTG CGG TTG GCA CTG GCG CAG AGC GGC ATA GAG CGC          98
Met Ala Leu Ser Leu Arg Leu Ala Leu Ala Gln Ser Gly Ile Glu Arg
 15                  20                  25                  30

GGT CCC ACA GCA TCA GCC CCC CAG GGG GAC CTG TTG TTC CTG CTG GAC         146
Gly Pro Thr Ala Ser Ala Pro Gln Gly Asp Leu Leu Phe Leu Leu Asp
                 35                  40                  45

AGC TCA GCC AGT GTG TCA CAC TAT GAG TTC TCA AGA GTT CGA GAG TTT         194
Ser Ser Ala Ser Val Ser His Tyr Glu Phe Ser Arg Val Arg Glu Phe
             50                  55                  60

GTG GGG CAG CTG GTG GCT ACG ATG CCT TTC GGA CCC GGG GCT CTG CGT         242
Val Gly Gln Leu Val Ala Thr Met Pro Phe Gly Pro Gly Ala Leu Arg
         65                  70                  75

GCT AGT CTG GTG CAC GTG GGC AGC CGG CCT CAC ACG GAG TTT ACT TTT         290
Ala Ser Leu Val His Val Gly Ser Arg Pro His Thr Glu Phe Thr Phe
     80                  85                  90

GAT CAG TAC AGT TCA GGC CAG GCC ATA CAG GAT GCT GTA CGT GTT GCG         338
Asp Gln Tyr Ser Ser Gly Gln Ala Ile Gln Asp Ala Val Arg Val Ala
 95                  100                 105                 110

CCC CAA CGT ATG GGT GAC ACC AAC ACG GGC CTG GCA CTG GCT TAT GCC         386
Pro Gln Arg Met Gly Asp Thr Asn Thr Gly Leu Ala Leu Ala Tyr Ala
                115                 120                 125

AAA GAA CAA TTA TTT GCT GAG GAA GCA GGT GCC CGG CTA GGG GTT CCC         434
Lys Glu Gln Leu Phe Ala Glu Glu Ala Gly Ala Arg Leu Gly Val Pro
            130                 135                 140

AAG GTA CTG GTG TGG GTG ACA GAT GGG GCC TCC AGT GAC TCT GTG GGC         482
Lys Val Leu Val Trp Val Thr Asp Gly Ala Ser Ser Asp Ser Val Gly
        145                 150                 155
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CCC CCT ATG CAG GAG CTC AAG GAC CTG GGT GTC ACC ATC TTC ATT GTC        530
Pro Pro Met Gln Glu Leu Lys Asp Leu Gly Val Thr Ile Phe Ile Val
    160                 165                 170

AGC ACT GGC CGA GGC AAC CTG TTG GAG CTG TTG GCA GCT GCC TCA GCT        578
Ser Thr Gly Arg Gly Asn Leu Leu Glu Leu Leu Ala Ala Ala Ser Ala
175                 180                 185                 190

CCG GCT GAG AAG CAC CTA CAC TTT GTG GAT GTG GAT GAC CTT CCT ATC        626
Pro Ala Glu Lys His Leu His Phe Val Asp Val Asp Asp Leu Pro Ile
                195                 200                 205

ATT GCC CGG GAG CTT CGG GGT GCC ATT ATT GAT GCG ATG CAG CCA CAT        674
Ile Ala Arg Glu Leu Arg Gly Ala Ile Ile Asp Ala Met Gln Pro His
            210                 215                 220

CAG CTT CAT GCT TCG GAG ATT CTG TCC AAT GGC TTC CGC CTG TCC TGG        722
Gln Leu His Ala Ser Glu Ile Leu Ser Asn Gly Phe Arg Leu Ser Trp
        225                 230                 235

CCG CCC CTG CTG ACA GCG GAC TCT GGT TAC TAC GTG CTG GAG TTG GTG        770
Pro Pro Leu Leu Thr Ala Asp Ser Gly Tyr Tyr Val Leu Glu Leu Val
    240                 245                 250

CCC AGT GGC AAA CTG GCA GCC ACA AGA CGC CAA CAG CTG CCC GGG AAT        818
Pro Ser Gly Lys Leu Ala Ala Thr Arg Arg Gln Gln Leu Pro Gly Asn
255                 260                 265                 270

GCT ACC AGC TGG ACC TGG ACC GAC CTC AAC CCA GAC ACA GAT TAC GAA        866
Ala Thr Ser Trp Thr Trp Thr Asp Leu Asn Pro Asp Thr Asp Tyr Glu
                275                 280                 285

GTA TCG CTG TTG CCG GAG TCC AAT GTG CGC CTC CTG AGG CCC CAG CAC        914
Val Ser Leu Leu Pro Glu Ser Asn Val Arg Leu Leu Arg Pro Gln His
            290                 295                 300

TTG CGA GTA CGC ACA CTG CAA GAG GAG GCA GGG CCA GAA CGC ATC GTC        962
Leu Arg Val Arg Thr Leu Gln Glu Glu Ala Gly Pro Glu Arg Ile Val
        305                 310                 315

ATC TCG CAT ACT AGG CCG CGC AGC CTC CGT GTA AGT TGG GCC CCC GCA       1010
Ile Ser His Thr Arg Pro Arg Ser Leu Arg Val Ser Trp Ala Pro Ala
    320                 325                 330

CTT GGC CCG GAC TCC ACT CTC GGC TAC CTT GTA CAG CTC GGA CCT CTG       1058
Leu Gly Pro Asp Ser Thr Leu Gly Tyr Leu Val Gln Leu Gly Pro Leu
335                 340                 345                 350

CAG GGC GGA TCC CTA GAA CAC GTG GAG GTG CCA GCT GGC CAG AAC AGC       1106
Gln Gly Gly Ser Leu Glu His Val Glu Val Pro Ala Gly Gln Asn Ser
                355                 360                 365

ACT ACC ATC CAG GGC CTG ACG CCC TGC ACC ACT TAC CTG GTG ACT GTG       1154
Thr Thr Ile Gln Gly Leu Thr Pro Cys Thr Thr Tyr Leu Val Thr Val
            370                 375                 380

ACT GCC GCC TTC CGC TCG GGC CGC CAG AGG GCG CTG TCG GCT AAG GCC       1202
Thr Ala Ala Phe Arg Ser Gly Arg Gln Arg Ala Leu Ser Ala Lys Ala
        385                 390                 395

TGT ACG GCA TCT GGC GAG CGG ATC CGT GTC CCG CAG GCC ATG CGG CCG       1250
Cys Thr Ala Ser Gly Glu Arg Ile Arg Val Pro Gln Ala Met Arg Pro
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    400                 405                 410

GAG GCC GGA CTG CGG GAG CCC TGACCTTCCT GCCTCGTCCA CCCGAGGGGC          1301
Glu Ala Gly Leu Arg Glu Pro
415                 420

CCTCTTCCCT AATCCAGTGA GAGAGGCACC GCTGCTCGTG GTTTTTCTTG TGGATGGAGT     1361

GGGGTGGGGA GATGGGGGTG CTGGTCCTAC CTTTGACCTG CGTAACTCCT CCGGTCGTTT     1421

CCCCACTGGT CATCACCGCC CTTGCCTGAC TCCCGGGAAA CCTGTAGCAA CCCTGGTAGC     1481

CTCACGCGCA ATGACAATCC TCTCCGGTTG CCAGTGGAGT TGAGCACACG GTGGTCCTTG     1541

GGCAACATTT GGCGAGGGGA TGGACAGTGT TTGAGGTCAG GTTGAGACCC AGGAGAAGCA     1601

TTCAGGAGAG GAGGCCACAG AGTTTTCTAC CTGTGCCAAA GACTGGGCCC TCTGGTGGCA     1661

AGGACTACAC ATGGCTTGGA GGAAATGTCC AGGACCCTTC AAGTCCTGCC TGTGCCTAGA     1721

AAGTGGGTAG GAGAAAGGGA GGAGAGAGTA GTGTAGGCAA GGTTCCCAAA GACTTCCTTA     1781

GGGCAAGGAA AGGTAGGGAG AGATACCGGG AGGCTGATGA TGTTGCCAAC TAGTTTTCAT     1841

CAAGATTTTC TGCCAGCCTG GAGGCCAGGA TCTGTCAGGG TCACTGACTC TGCCTTCCTG     1901

CCCAGGACCT GCACTGGGCC CCGCTATCAG TGCGGGGGGG GGGGTGCAGA GTCTTCACAG     1961

GAATGGGGGA TGAGACCTTG GCATGTAGTA CATTGGGGAT AGGAGAGCCC TGCCGTGACA     2021

GACTTACAGG GAGTCTCCTG CTTTAGTGTA GGGAGCAAGG TGACATGCAG GTGGGCTACC     2081

TCCTGTCATC ACTACTGCCC TGGGGCATCT GACAGATACC TAAGGGTAGT CAGGAACAGG     2141

CTTCCTCTCC AGTCCCTATG TACGCAGAGC CCCTCCTCCC CAGAACCTCT TGCCCTAACC     2201

TAAGCTTACT CCATCTCTCT TCCCCACTAA TGACCCAGAC TCTAACAATA ATACAGTAAG     2261

CCAGATGTAA ACTGTGAAGT CGAC                                            2285
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(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 421 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

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Pro Ala Thr Pro Gly Ala Met Leu Phe Trp Thr Val Leu Ser Met Ala
 1               5                  10                  15

Leu Ser Leu Arg Leu Ala Leu Ala Gln Ser Gly Ile Glu Arg Gly Pro
            20                  25                  30

Thr Ala Ser Ala Pro Gln Gly Asp Leu Leu Phe Leu Leu Asp Ser Ser
        35                  40                  45

Ala Ser Val Ser His Tyr Glu Phe Ser Arg Val Arg Glu Phe Val Gly
    50                  55                  60
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